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POLYMORPHONUCLEAR LEUKOCYTE ACTIVATOR
[Takeikaku hakkekyu kasseikazai]

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1. Name of this Invention

POLYMORPHONUCLEAR LEUKOCYTE ACTIVATOR

2. Claim(s)

[1] Polymorphonuclear leukocyte activator comprising histamine H₁ acceptor antagonist and/or histamine H₂ acceptor antagonist as a valid substance.

[2] Periodontal disease prevention/treatment agent containing a polymorphonuclear leukocyte activator according to Claim 1.

3. Detailed Explanation of this Invention

[Technological Field]

This invention generally pertains to a polymorphonuclear leukocyte activator and is particularly associated with a medicinal agent capable of preventing and curing various infection-caused diseases by activating polymorphonuclear leukocytes having a role in the defense mechanisms against infections caused by bacteria, etc.

[Conventional Technology]

To cure infection-caused diseases caused by bacteria and the like, various antibiotics are used. For example, an antibiotic such as tetracycline or a germicide such as metronidazole is used as a medicine for treating periodontal diseases known to be bacteria-caused.

*Numbers in the margin indicate pagination in the foreign text.

Also, as one of human defense functions to bacteria-related infections, the phagocytosis function of polymorphonuclear leukocytes that phagocytizes bacteria is known.

[Problems to Be Solved by this Invention]

The treatment of infection-related diseases using an antibiotic may not provide sufficient effect. For example, when treating periodontal diseases, which are infections caused by bacteria usually present in the human mouth, antibiotics or germicides can temporarily improve the symptoms by reducing germs by administering a prescribed medicine. However, once the administration period ends, the bacteria usually present in the human mouse are multiplied again to create recurrence of symptoms. Furthermore, by administering an antibiotic or germicide over a long period in order to prevent the symptom recurrence, the production of bacteria resistant to the prescribed medicine or formation of disease by alternate bacteria such as *Candida* will be highly likely.

[Method to Solve the Problems]

By focusing on polymorphonuclear leukocytes functioning to phagocytize bacteria, which is a key factor in suppressing the onset of periodontal disease among natural immunogenic functions present in human body, the developers of this invention believed that, by activating the polymorphonuclear leukocytes or normalizing the polymorphonuclear leukocytes having low phagocytosis capacity, pathogenic bacteria can be sterilized and removed from the infection

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lesion, subsequently allowing prevention of or curing various infections. After diligent investigation on histamine widely known as a kind of inflammation mediator, they discovered that the phagocytizing activity of polymorphonuclear leukocyte was suppressed by histamine, and moreover, a histamine acceptor antagonist could recover the phagocytizing activity of polymorphonuclear leukocyte, which would subsequently lead to cure infections such as periodontal diseases.

The object of this invention is to provide a polymorphonuclear leukocyte activator comprising a histamine H₁ acceptor antagonist and/or a histamine H₂ acceptor antagonist as active ingredients.

The histamine H₁ acceptor antagonist of this invention is not particularly restricted. Examples are amino alkyl ether type diphenhydramine and doxylamine, ethylene diamine type pyrilamine, imidazoline type antazoline, henotiadine type promethazine or arimemazine, homopiperadine type homochlorocycrizine, propyl amine type chrolopheniramine, piridoidene type phenhydramine, cycloheptane type cyproheptadine, and the like. In addition, the histamine H₂ acceptor antagonist is not particularly limited in this invention. Examples are brimamid, metiamide, cimetidine, ranitidine, and the like. These histamine receptor antagonists may be used individually or combined.

The function of activating polymorphonuclear leukocytes and the curing effect of infection-caused diseases were examined on the

typical histamine receptor antagonists used as active ingredients of this invention. The test results are described below:

(a) Phagocytizing function of polymorphonuclear leukocyte diminished by histamine and effect of histamine receptor antagonist applied for recovering diminished bacteria-phagocytizing function:

Blood was taken from the heart of a male Hartley guinea pig (body weight = approx. 500 g), from which leukocytes were collected using a Monopoly-separation solution (product of Flow Co.). Then, polymorphonuclear leukocytes were separated using a Percoli specific gravity centrifugal method, washed with HBSS (Hanks' balanced salt solution), and tested with a Tripan blue exclusion test so as to confirm that at least 95% of cells were alive. The collected cells were dispersed in HBSS and used as polymorphonuclear leukocyte samples.

Separately, zymosan was reacted in a carbonic acid buffer solution (pH 9.1) with 0.5% FITC (fluorencinizo thiocyanate) for 2 hours and dispersed in HBSS. Then, after guinea pig serum was added for 1 ml per 2.5×10^4 pieces of prepared zymosan, the sample mixture was incubated at 37°C for 30 min. to promote opsonization using a complement.

The above-mentioned polymorphonuclear leukocyte (final concentration: 1.6×10^6 pieces/ml) and medicine were preincubated at 37°C for 20 min. in a test tube, to which the above-mentioned zymosan (final concentration: 2.4×10^7 pieces/ml) was added to cause

phagocytosis at 37°C and 0°C for 20 min. Next, the average strength of fluorescence per 1000 cells was acquired using a flow cytometer (product of BBCTON Dickinson). The gap of measured strengths between 37°C and 0°C was considered as a phagocytic activity.

The tested medicines were histamine hydrochloric acid salt, hydrochloric acid pyrilamine as a typical H₁ receptor antagonist, and hydrochloric acid cimetizine as a typical H₂ receptor antagonist.

The following method was used to evaluate the medicines. First, the histamine-caused suppression of phagocytic activity of polymorphonuclear leukocyte not treated by a medicine was confirmed by providing a 1 mM histamine. Next, after the phagocytic activities of polymorphonuclear leukocytes were measured by providing histamine and various antagonisms (10, 100, 1000 μM), the recoveries of phagocytic activities were examined. The obtained results are shown in Figs. 1A and 1B. The pyrilamine used as an H₁ receptor antagonist and hydrochloric acid cimetizine used as an H₂ receptor antagonist both could recover the phagocytic activities to specific degrees depending on the dosages. Antagonists at the level of at 100 μg/ml could provide the phagocytic activities equal to the activity level of comparison subject.

(b) Effect of histamine receptor antagonist to the naturally caused dog gingivitis:

A dog feed liquefied with water was given to a female Beagle (body weight = approx. 4 Kg) for a year to naturally form gingivitis caused by the usual bacteria present in the mouse.

1 ml of medicine was injected per lesion inside the inflammatory gum groove once a day using a root canal syringe under the thiopental administration. The used medicines were physiological salt aqueous solutions (containing 10% of ethanol) of maleic acid chloro pheniramine as an H₁ receptor antagonist and hydrochloric acid cimetidine as an H₂ receptor antagonist. The concentration of each solution was 0.1%. A physiological salt aqueous solution used as a control and three kinds of medicines were allocated to the specific areas of dog jaw sectioned by dividing the entire jaw into upper/lower and left/right four treatment sections. The treatment was continued for 5 days, wherein the degree of inflammation was evaluated on the first day, third day and fourth day of the test under the administration of halothane-inhalation anesthesia. The degree of inflammation was evaluated based on the amount of fluid coming out from the gum groove (periotron value), as this amount, which increases with the progression of gum inflammation, is effective for measuring gum inflammation. The measured results are shown in Figs. 2A - 2C. The results revealed that hydrochloric acid cimetidine, an H₂ receptor antagonist, significantly suppressed inflammation, while hydrochloric acid ranitidine showed the propensity of inflammation suppression. /171

(c) Effect of histamine receptor antagonist in curing severe gingivitis of a dog experimentally formed with a ligature:

Teeth of a female Beagle (body weight = approx. 4 Kg) were flossed once a day for a year in order to establish healthy gum. Then, a surgical thread used as a ligature was wound around the bottom of the teeth on the experimenting gum under the administration of pentobarbital anesthesia. As a result, strong gum inflammation was formed a week later. Then, the same set of medicines described in (b) were applied to the inflammatory area. The used medicines were hydrochloric acid pyrilamine, which was an H₁ receptor antagonist, hydrochloric acid cimetidine, which was an H₂ receptor antagonist, and indomesacine, which was a non-steroid type anti-inflammatory medicine used as a control. Those medicines were prepared respectively as 0.8% (20 mM), 0.5% (20 mM), and 0.07% (2 mM) physiological salt aqueous solution (including 10% ethanol), and injected into the gum groove for 1 ml per gum section once a day. The fluid coming out from the gum was measured prior to the onset of gum inflammation, initial medication date, second day, fourth day and sixth day in the same way as described in (b).

Figures 3A - 3D show the measurement results.

The inflammation was significantly suppressed on the fourth day by hydrochloric acid pyrilamine (H₁ receptor antagonist), on the sixth day by hydrochloric acid cimetidine (H₂ receptor antagonist), and fourth day and sixth day by indomesacine (non-steroid type anti-

inflammatory medicine). The area not treated by any of the medicines did not show any improvement to the inflammation.

As shown in experiment results (a) - (c), both the histamine H₁ receptor antagonist and histamine H₂ receptor antagonist recovered the phagocytizing activity of polymorphonuclear leukocyte weaken by histamine. Moreover, they improved the gingivitis which was an inflammation disease caused by the bacteria normally present in the mouth. Therefore, histamine receptor antagonists are useful as polymorphonuclear leukocyte activators. Furthermore, in addition to periodontal diseases, the polymorphonuclear leukocyte activator of this invention can be used for curing many infections causing inflammations and is especially effective for opportunistic infections to which antibiotics and germicides are ineffective.

With an appropriate molding agent, carrier, diluting agent, etc., the polymorphonuclear leukocyte activator of this invention can be formed as orally or non-orally administrative tablet, capsule, particle, powder, injection liquid agent injected to a gum groove, ointment, medicine-impregnated soluble stick, plaster, pap agent, injection agent, suppository, and the like. These formulations can be prepared by the conventional methods. For example, an orally administrative medicine can be produced by appropriately combining a histamine receptor antagonist with a molding agent (e.g., starch, mannitol, lactose, etc.), bonding agent (e.g., carboxy methyl cellulose sodium, hydroxy propyl cellulose, etc.), destruction agent

(e.g., crystal cellulose, carboxy methyl cellulose calcium, etc.), lubricant (e.g., talc, stearic acid magnesium, etc.), fluidity enhancer (e.g., light-weight anhydrous silicate, etc.), and the like. Moreover, to prepare an external-use agent (e.g., ointment, pap agent, etc.), conventionally known base agents (e.g., Vaseline, lanoline, paraffin, silicone, plant oil, etc.) can be used.

The amounts of H₁ receptor antagonist and/or H₂ receptor antagonist in the polymorphonuclear leukocyte activator of this invention may be any quantity as long as they effectively activate polymorphonuclear leukocytes. However, for internally administrative medicines, the amount is preferably 1 mg - 1000 mg/day, while the amount of an external-use medicine is preferably 0.01 - 60 wt. %. Furthermore, other medicines combinable with H₁ receptor antagonist and H₂ receptor antagonist may be added simultaneously. Especially, the combination of polymorphonuclear leukocyte activator of this invention and antibiotics is effective.

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[Embodiments of this Invention]

Hereafter, this invention will be explained in detail by referring to the embodiments of this invention. Note that this invention is not limited to these embodiments.

Embodiment 1:

Periodontal disease treatment agent

Hydrochloric acid pyrilamine	0.1
Hydrochloric acid cimetidine	0.1
Refined water	Appropriate amount

Total	100 (wt. %)

The above-mentioned liquid agent is injected into the gum groove using a root canal syringe.

Embodiment 2:

Periodontal disease treatment agent

Hydrochloric acid pyrilamine	0.1
Hydrochloric acid ranitidine	0.1
Hydroxy ethyl cellulose	1.0
Refined water	Appropriate amount

Total	100 (wt. %)

The above-mentioned liquid agent is injected into the gum groove using a root canal syringe.

Embodiment 3:

Periodontal disease treatment agent

Hydrochloric acid ranitidine	0.1
Acetic acid tocopherol	5.0
N lauroyl arginine ethyl ester	0.5
Ethanol	Appropriate amount

Total	100 (wt. %)

The above-mentioned composition is added in 10 times water to form an emulsion used as a mouth rinse.

Embodiment 4:

Hemorrhoid treatment agent

Hydrochloric acid cimetidine	0.2
Quercetin	0.1
Hydrochloric acid chlorohexidine	0.05
Cocoa fat	Appropriate amount
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Total	100 (wt. %)

The above-mentioned composition is melted with heat and mixed and molded to a round-tip cone suppository shape.

Embodiment 5:

Skin disease treatment agent

Hydrochloric acid pyrilamine	0.1
Hydrochloric acid ranitidine	0.1
Glycyl retin acid	0.02
Acetic acid tocopherol	0.1
White Vaseline	Appropriate amount
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Total	100 (wt. %)

The above-mentioned ointment can be applied to the affected skin area.

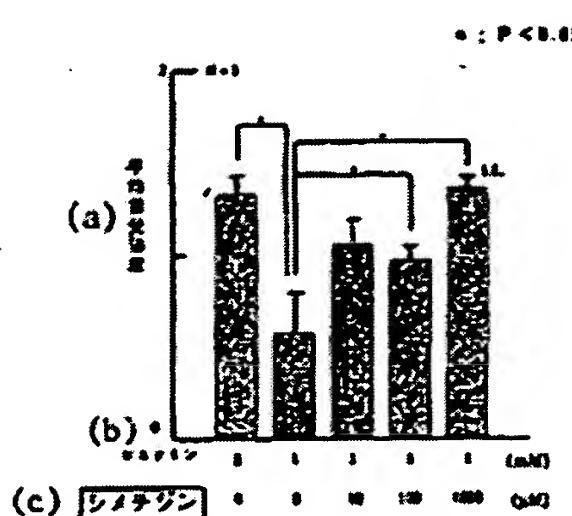
[Effect of this invention]

The method based on this invention can provide effective medicines capable of treating hardly curable infection-caused diseases formed by diminished activities of polymorphonuclear leukocytes due to bacterial infection, such as periodontal diseases, athlete's foot, hemorrhoid, etc. and opportunistic infection.

4. Simple Explanation of the Figures

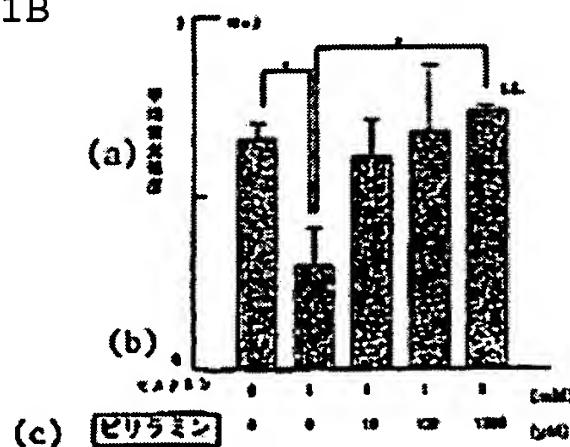
Figures 1A and 1B are diagrams showing the effects of hydrochloric acid cimetidine and hydrochloric acid pyrilamine to the polymorphonuclear leukocyte phagocytosis (average fluorescent strength) lowered by histamine. Figures 2A, 2B, and 2C are diagrams illustrating the effects of physiological salt water, ranitidine, and hydrochloric acid cimetidine provided to the amount of fluid produced from gum groove (periotron value) due to naturally formed gingivitis. Figures 3A - 3D are diagrams showing the activities of physiological salt water (control), hydrochloric cimetidine, hydrochloric pyrilamine, and indomesacine to the amount of fluid produced from gum groove (periotron value) due to gingivitis of a dog caused by a ligature.

Figure 1A



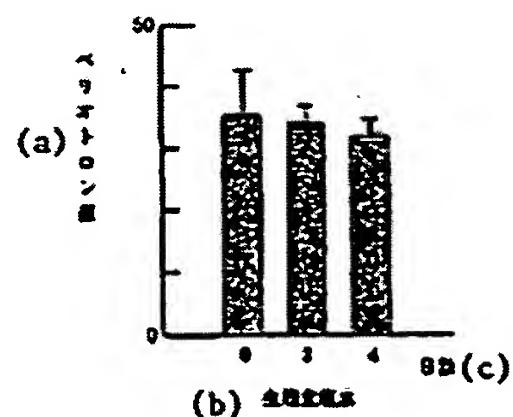
Key: (a) Ave. Fluorescent strength; (b) Histamine; (c) Cimetidine.

Figure 1B



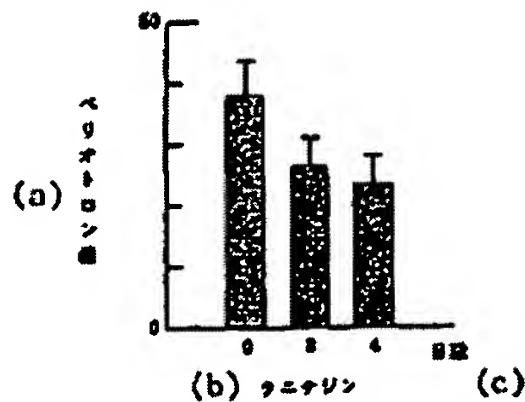
Key: (a) Ave. Fluorescent strength; (b) Histamine; (c) Pyriramine;

Figure 2A



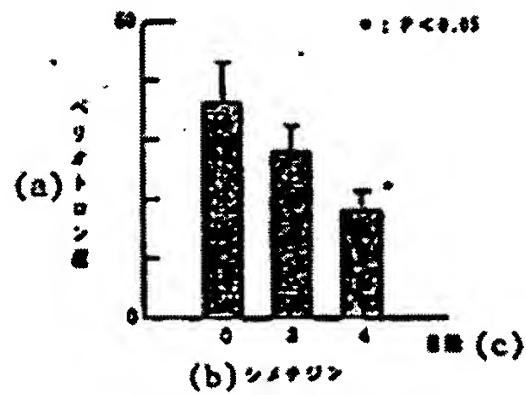
Key: (a) Periotron value; (b) Physiological salt water; (c) Day count.

Figure 2B



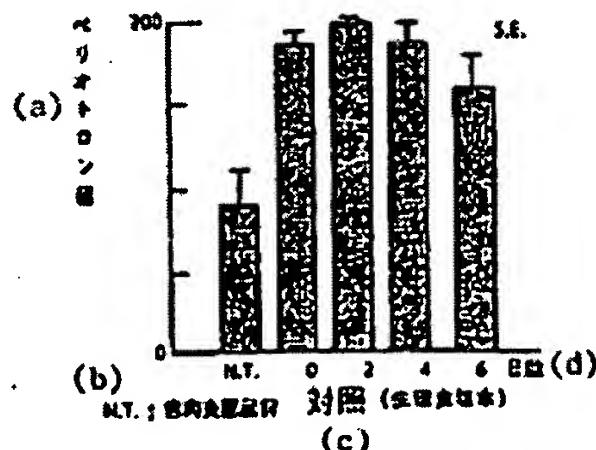
Key: (a) Periotron value; (b) Ranitidine; (c) Day count.

Figure 2C



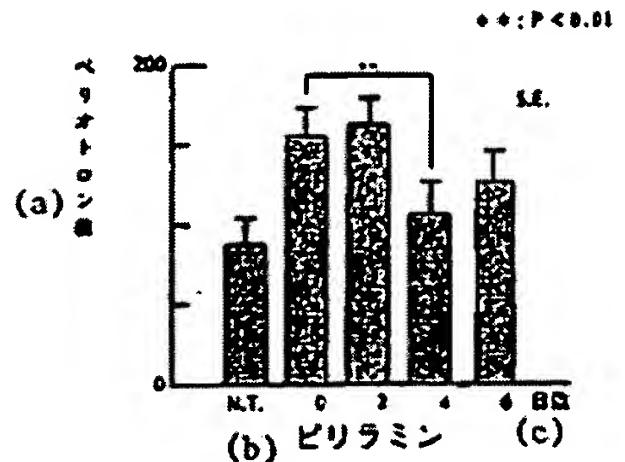
Key: (a) Periotron value; (b) Cimetidine; (c) Day count.

Figure 3A



Key: (a) Periotron value; (b) N.T.: Before onset of gingivitis; (c) Comparison (phy. Salt water); (d) Day count.

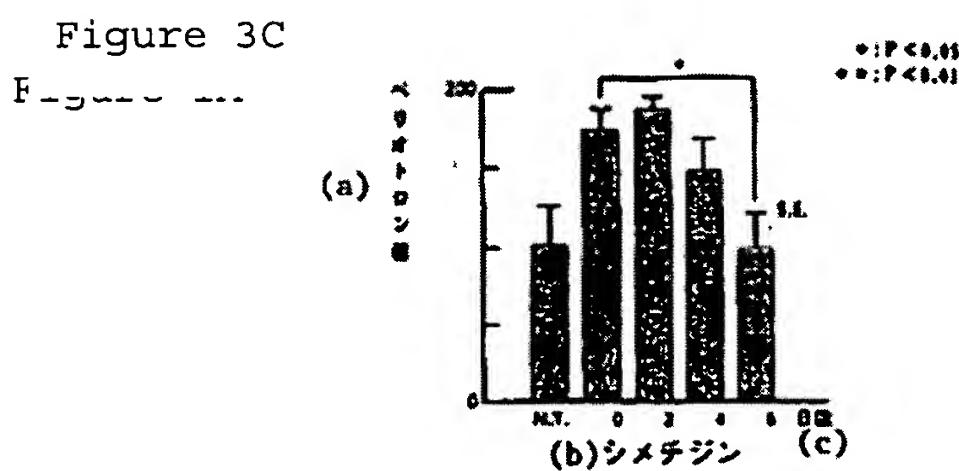
Figure 3B



Key: (a) Periotron value; (b) Pyriramine; (c) Day count

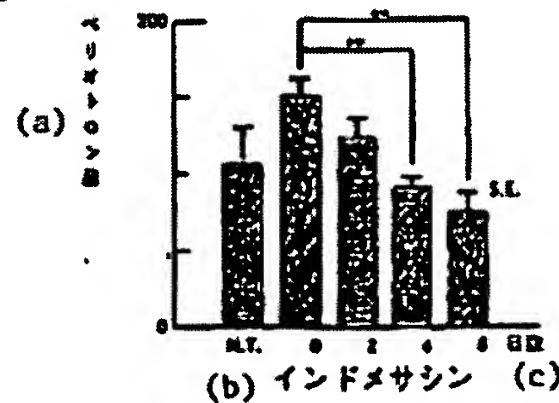
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Figure 3C



Key: (a) Periotron value; (b) Cimetidine; (c) Day count.

Figure 3D



Key: (a) Periotron value; (b) Indomesacine; (c) Day count.